Enzymatic Assay of SARCOSINE DEHYDROGENASE
(EC 1.5.99.1)

PRINCIPLE:

$$\text{Sarcosine} + H_2O + \text{PMS} \xrightarrow{\text{Sarcosine Dehydrogenase}} \text{Glycine} + \text{HCHO} + PMSH_2$$

$$2\ PMSH_2 + \text{NTB} \longrightarrow 2\ \text{PMS} + \text{Diformazan}$$

Abbreviations used:
PMS = Phenazine Methosulfate
NTB = Nitro Blue Tetrazolium
$PMSH_2$ = Phenazine Methosulfate (Reduced Form)

CONDITIONS: T = 37°C, pH = 7.5, A_{570nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer with 500 mM Sarcosine and 0.5% (v/v) Triton[1], X-100, pH 7.5 at 37°C (Substrate Solution)
(Prepare 25 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, Sarcosine, Free Base, Sigma Prod. No. S-9881, and Triton[1] X-100, Sigma Stock No. X-100. Adjust to pH 7.5 at 37°C with 1 M KOH.)

B. 0.01% (w/v) Phenazine Methosulfate and 0.1% (w/v) Nitro Blue Tetrazolium Color Reagent (PMS-NBT)
(Prepare 5 ml in deionized water using Phenazine Methosulfate, Sigma Prod. No. P-9625 and Nitro Blue Tetrazolium, Sigma Prod. No. N-6876.)

C. 300 mM Hydrochloric Acid Solution (HCl)
(Prepare 25 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)

D. 50 mM Potassium Phosphate Buffer, pH 7.5 at 37°C (Enzyme Diluent)
(Prepare 25 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 37°C with 1 M KOH.)

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REAGENTS: (continued)

E. Sarcosine Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.013 - 0.025 units/ml of Sarcosine Dehydrogenase in Reagent D. Immediately prior to starting the reaction, equilibrate to 37°C.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	Test	Blank
Reagent A (Substrate Solution)	0.50	0.50
Reagent B (PMS-NBT)	0.10	0.10

Mix by swirling and equilibrate to 37°C. Then add:

Reagent E (Enzyme Solution)	0.50	------

Immediately mix by swirling and incubate for exactly 15 minutes at 37°C. Then add:

Reagent C (HCl)	3.00	3.00
Reagent E (Enzyme Solution)	------	0.50

Mix by swirling and transfer to suitable cuvettes. Record the A_{570nm} for both the Test and Blank in a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{570nm}\ \text{Test} - A_{570nm}\ \text{Blank})(4.1)(df)}{(15)\ (20.1)\ (0.5)}$$

4.1 = Total volume (in milliliters) of assay
df = Dilution factor
15 = Time of assay (in minutes) as per the Unit Definition
20.1 = Millimolar extinction coefficient of diformazan at 570nm
0.5 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

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CALCULATIONS: (continued)

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will convert 1.0 µmole of sarcosine to glycine and formaldehyde per minute at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.10 ml reaction mix, the final concentrations are 45 mM potassium phosphate, 227 mM sarcosine, 0.2% (v/v) Triton X-100, 0.0009% (w/v) phenazine methosulfate, 0.009% (w/v) nitro blue tetrazolium, and 0.0065 - 0.0125 unit sarcosine dehydrogenase.

NOTES:

1. Triton X-100 is a registered trademark of Union Carbide.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.